that the phosphorylation of residue S2849 leads to the formation of an arginine claw that is absent in the non-phosphorylated protein. This finding at least partly elucidates the phenotypes stemming from several disease-linked human mutations in DP. We are currently determining if R2834H, a mutation that has been linked to arrhythmogenic right ventricular cardiomyopathy, disrupts the claw structure; we are also examining the effects of methylation of R2834, which has recently been shown to control the extent of phosphorylation. This work will illuminate the structural mechanisms by which DP adhesion is ultimately controlled.

1957-Pos Board B94

The Role of Higher-Order SPOP Oligomers for Localization to Cellular "Bodies" and Ubiquitination Activity

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Light microscopically detectable, non-membrane bound cellular ''bodies'' are large protein assemblies with liquid-like properties, but the biophysical basis of their formation is unclear. Weak, multivalent protein interactions can result in higher-order complexes and can enable the formation of cellular bodies. The inherent size heterogeneity of higher-order complexes renders them difficult to characterize biophysically. As a result, their size distributions remain largely unquantified, limiting molecular insight into their biological functions. We report a novel mechanism governing cellular body formation of the Speckle-type POZ protein (SPOP), which was recently identified as tumor suppressor, is a ubiquitin ligase substrate adaptor that localizes to nuclear puncta. We demonstrate that its cellular localization is dependent upon the ability of SPOP to form higher-order homo-oligomers through indefinite self-association, mediated by two distinct oligomerization domains. Furthermore, in vitro ubiquitination of substrates is enhanced through higher-order self-association of SPOP, suggesting that SPOP puncta are hotspots of substrate ubiquitination. One of SPOP's domains dimerizes with nanomolar affinity yielding stable SPOP dimers as ''building blocks'' for indefinite self-association, while the other domain dimerizes with micromolar affinity, rendering SPOP oligomers highly dynamic. Together, this results in isodesmic self-association, in which each addition of a dimer occurs with the same affinity, independent of the oligomer size. From this model, we describe the size distribution of SPOP oligomers, providing for the first time a quantitative analysis of protein assemblies participating in the formation of cellular bodies. Mutations within both oligomerization domains have been observed in a variety of cancers, supporting our conclusion that SPOP self-association is important for its biological function. We propose that dynamic, higher-order protein self-association is a general mechanism underlying the formation of cellular bodies, which may serve as switches to fine-tune signaling cascades.

Ribosomes and Translation

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Towards a Whole-Cell Model of Ribosome Biogenesis: Kinetic Modeling of SSU Assembly

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Ribosome biogenesis is a coordinated process involving the hierarchical association of 21 proteins to the 16S rRNA in the small subunit and 33 proteins to the 5S and 23S rRNAs in the large subunit. The process is further complicated by effects arising from the intracellular environment such as molecular crowders and the location of ribosomal operons within the cell. We report on our progress on the construction of a whole-cell model of ribosome biogenesis. Here we describe a detailed kinetic model accounting for the association of 18 of the 20 ribosomal proteins to the 16S rRNA to form the small subunit in vitro. Construction of the model is guided by the Nomura map of thermodynamic protein binding dependencies as well as kinetic cooperativity data. The complex chemical reaction network is simplified to 180 distinct assembly intermediates by removing infrequently used species. The 5′-central-3' binding order proposed in the literature is reproduced and an alternate assembly pathway, 5′-3′-central, is predicted which accounts for 30% of the total reaction flux. Biologically relevant assembly intermediates are identified and compared to intermediates observed using cryo-electron microscopy. Integration of this assembly model into an in vivo, spatially resolved whole-cell model of biogenesis accounting for the transcription and translation of ribosomal components using realistic cellular geometry will be discussed.

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A Structural Model of the Ribosome-Bound Protein Insertase YidC Reveals Lateral Translocation of the Nascent Chain

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The integration of membrane proteins into the cytoplasmic membrane of bacteria usually occurs co-translationally. The universally conserved YidC protein mediates this process either individually as a membrane protein insertase, or as a membrane protein chaperone in concert with the canonical protein-conducting channel, the SecY complex. However, little is known about the structural basis of YidCs interaction with ribosome, and its co-translational insertion activity. Here, we present a structural model of YidC based on evolutionary co-variation analysis, lipid versus protein exposure and molecular dynamics simulations. The model suggests a distinct arrangement of the conserved five transmembrane domains and an amphipathic helical hairpin between TM2 and TM3 on the cytoplasmic surface of the bilayer. The model was used for docking into a cryo-electron microscopy reconstruction of a translating YidC-ribosome complex carrying the YidC substrate FOc. This structure revealed how a single copy of YidC interacts with the ribosome at the ribosomal tunnel exit and suggests a site for membrane protein insertion at the YidC protein-lipid interphase. This site was confirmed by chemical crosslinking of FOc to TM3 of YidC. Together, these data suggest a mechanism for the co-translational mode of YidC-mediated membrane protein insertion.

1960-Pos Board B97

RNA Structural Modulation in the Heart of the Ribosome

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DEAD-box RNA helicase DbpA is one of the RNA maturation factors that *E. coli* employs during its ribosome assembly process. DbpA binds tightly and specifically to hairpin 92 of the 23S ribosomal RNA which is located in the peptidyl transferase center. Therefore, DbpA is implicated in RNA structural rearrangement in a ribosome region that is crucial for cell survival. When the helicase inactive R331A DbpA construct is expressed in *E. coli* cells, a 45S particle accumulates. This particle is a misassembled intermediate of the large ribosome subunit. It is not known if the 45S misassembled particle rearranges inside the cell and forms the active 50S large ribosome subunit, or if the resulting RNA structural misfolding is so severe that the 45S particle is designated by the cell for degradation. To understand the fate of the 45S particle in the cell, the ability of the 45S particle to form a native 50S subunit is tested by pulse chase. First, in the cell expressing R331A DbpA and lacking the wild type DbpA from their genome, RNA is labeled with [5,6-^{3}H] uridine for a specific amount of time, and then transcription of new RNA is stopped by the addition of rifampicin. Cell culture aliquots are obtained at a series of time points after stopping the transcription of new RNA, and ribosomal profile analyses are performed using sucrose gradient ultracentrifugation. The ribosome profile experiments demonstrate that the conversion of the 45S intermediate to the 50S large subunit particle does occur in the cells. The conversion rate of the 45S particle to the 50S particle is currently being measured.

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Simulating Ribosome Dynamics and tRNA Translocation

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With advances in structure determination and continued growth in high-performance computing (HPC), molecular dynamics (MD) simulations can now be employed to study large-scale conformational rearrangements in molecular machines, such as the ribosome. In the cell, proteins are synthesized by the joint action of the ribosome and transfer RNA (tRNA) molecules, enabling messenger RNA (mRNA) to be translated into peptides. In the elongation cycle of translation, tRNA molecules and the associated mRNA move between binding sites, a process known as tRNA translocation. During translocation, tRNA movement (~20-50 Å) is coupled to large-scale collective rotations in the ribosomal subunits. In order to better understand the physical relationship between these rotations and tRNA displacements, we use MD simulations that employ a simplified description of the energetics, which elucidate the role of sterics, and molecular flexibility during tRNA translocation. For the ribosome, we construct forcefields for which each experimentally-derived

configuration is a potential energy minimum. Using these models, we are able to simulate spontaneous tRNA translocation events and identify robust aspects of the dynamics. We find that detailed steric interactions are a dominant contributor to tRNA translocation dynamics. These results provide a framework for understanding the interplay between structure and dynamics, and suggest strategies to experimentally modulate the physical-chemical features that govern ribosome function.

1962-Pos Board B99

Single-Molecule Profiling of Ribosome Recoding Phenomena

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Messenger RNA (mRNA) sequence is central to translational control, with special sequences and secondary structures regulating translational dynamics. Shine-Dalgarno sequences, mRNA hairpins and pseudoknots, as well as nascent peptide-ribosome interactions, are known to pause or stall the ribosome. These stimulatory elements may lead to kinetic branchpoints during elongation and induce recoding events, wherein the ribosome is shunted into alternative pathways that result in either changes in reading frame or the bypassing of a region of the mRNA. Here, we present single-molecule fluorescence methods with zero-mode waveguides (ZMWs) to profile directly the translational rates of thousands of single ribosomes with codon resolution, illuminating the underlying dynamic mechanisms of recoding events. We investigated two recoding events: the −1 frameshifting in the dnaX gene and the ribosome bypassing of a 50 nucleotide untranslated region in gene60 of T4 phage. We observed multiple pathways induced by the stochastic interaction of the ribosome with the stimulatory elements; the ribosomes that undergo recoding in both frameshifting and bypassing are characterized by a pause in the rotated state. Such paused states allow unusual events in elongation and may be a central feature of translational control.

1963-Pos Board B100

Ribosome Assisted GTP Hydrolysis by EF-Tu - Mechanism and the Role of Asp21

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The elongation factor Tu (EF-Tu) is a member of the translational GTPase superfamily, whose GTPase activity is stimulated by the ribosome. Recently we elucidated the GTPase mechanism of EF-Tu using computer simulations (*Ram Prasad et al. PNAS USA, 110, 20509, (2013)*) and concluded that His84 of switch II region acts mainly in an indirect way (i. e., it neither acts as a general base nor stabilizes the TS in a major way). Additionally, we also concluded that although the proton transfer step occurs through an additional water molecule it does not constitutes the rate-limiting barrier. These computational predictions are further confirmed by a recent mutational and biochemical study (*Maracci et al. PNAS USA, doi:10.1073/pnas.1412676111, (2014)*). This work found that a mutation of Asp21 in the P loop also hampers the GTPase activity of EF-Tu. This observation suggested that the catalytic effect is modulated by the nature of amino acid side chain — thus we obtain a support to our proposal of allosteric control by the preorganization of the p-loop. However, in order to identify conclusively the origin of the observed mutational effects, and thereby the actual role of D21 on the GTPase activity of EF-Tu, it is essential to move to a quantitative structure function analysis rather than mere qualitative arguments. Thus, we conducted a computational study aimed at examining and quantifying the molecular origin of the catalytic effect of Asp21 on the GTPase activity of EF-Tu.

1964-Pos Board B101

Using Hydroxyl Radical Footprinting to Observe Ribosome Assembly Intermediates in vivo

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The assembly of the E. coli ribosome small subunit has been widely studied and characterized in vitro. Despite this, ribosome biogenesis in living cells remains poorly understood. This is a very complex process in which an rRNA is transcribed, folded, cleaved, and modified, while also binding with 20 different proteins. Very little is known about how the tertiary structure of the ribosomal RNA changes during assembly. There are a number of structure-probing methods that can be used to study rRNA in vivo, but virtually all of them lack the time resolution necessary to study a process like ribosome synthesis, which is completed within a few minutes.

Hydroxyl radical footprinting can be used to probe in vivo rRNA structure. The hydroxyl radicals which probe the rRNA can be produced in milliseconds using synchrotron X-rays. With this technique it is possible to examine ribosome assembly with meaningful time resolution. The hydroxyl radicals cleave the RNA backbone in solvent accessible regions, giving cleavage patterns that reflect regions of flexibility and rigidity within an RNA.

For the purpose of examining ribosome assembly, it is nascent ribosomes that are of interest, not pre-existing ribosomes that are already assembled. Therefore, the nascent ribosomes must be isolated from the background of pre-existing ribosomes. It has been shown that cells can take up labeled nucleosides that have been added to their growth media and incorporate them into nascent RNA transcripts. These can then be isolated using affinity methods. Once the nascent, assembling rRNA has been isolated, it can be analyzed by primer extension. The reverse transcriptase terminates at the cleavage sites. The cDNA fragments are then able to be analyzed by either slab gel, capillary electrophoresis, or high-throughput sequencing methods.

1965-Pos Board B102

Exploring the Mechanism of Dhh1-Mediated Translational Repression

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The yeast protein Dhh1, along with its orthologs in higher eukaryotes, have long been implicated in the regulation of protein expression by activation of mRNA decapping and subsequent degradation. More recent studies have argued that repression of protein production by Dhh1 occurs via a cap-independent mechanism.

Through a combination of in vitro and in vivo studies using reporter assays and protein tethering, we show that translational repression by Dhh1 occurs concurrently with the formation of mRNA species ''over-loaded'' with ribosomes - or polyribosomes. Through these studies, we are further able to establish a minimal functional unit of Dhh1 - comprising only of the two central RecA domains - which is capable of engendering general translational repression.

We are currently complementing these studies with high-throughput ribosome profiling analysis to ascertain the nature of Dhh1-mediated translational control across the genome with nucleotide resolution. These experiments will allow us both to look at endogenous genes affected by deletion or overexpression of Dhh1 and to look at reporter constructs again in the presence of tethered Dhh1 protein.

1966-Pos Board B103

Extra-Coding Characteristics of hERG mRNA are Essential for Channel Function

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The KCNH2 gene encodes the hERG protein, the alpha subunit of the rapid delayed rectifying potassium channel. This potassium channel plays an essential role in cardiac repolarization, and malfunction of this channel caused by mutation leads to Long QT Syndrome, type 2 (LQT2). LQT2 causes increased repolarization time in the heart, leading to ventricular arrhythmias, syncope and sudden death. There are over 600 documented mutations in KCNH2 associated with Long QT syndrome, with more mutations being reported regularly. These mutations occur throughout the length of the gene, without definitive mutational hotspots.

While much investigation has been done to characterize the impact that these mutations have on the hERG channel function, little investigation has been focused on what causes the hERG channel to be intolerant to mutation. Our hypothesis is that ''extra-coding'' characteristics on the mRNA level, such as GC content, rare codon usage and mRNA structure play a critical role in determining correct protein synthesis for the hERG channel, and that mutational changes that disrupt these characteristics lead to Long QT Syndrome. To investigate this hypothesis first, hERG SNPs will be analyzed to both identify trends in disease-causing SNPs, and to find differences between disease-causing and benign SNPs such as changes in local GC content or disruption of codon usage frequency. Secondly, using a codon-modified hERG mRNA with decreased rare codon usage, GC content and CpG islands when compared to native hERG mRNA, the role of mRNA structure and ribosomal movement in determining secondary and tertiary protein structure will be investigated.